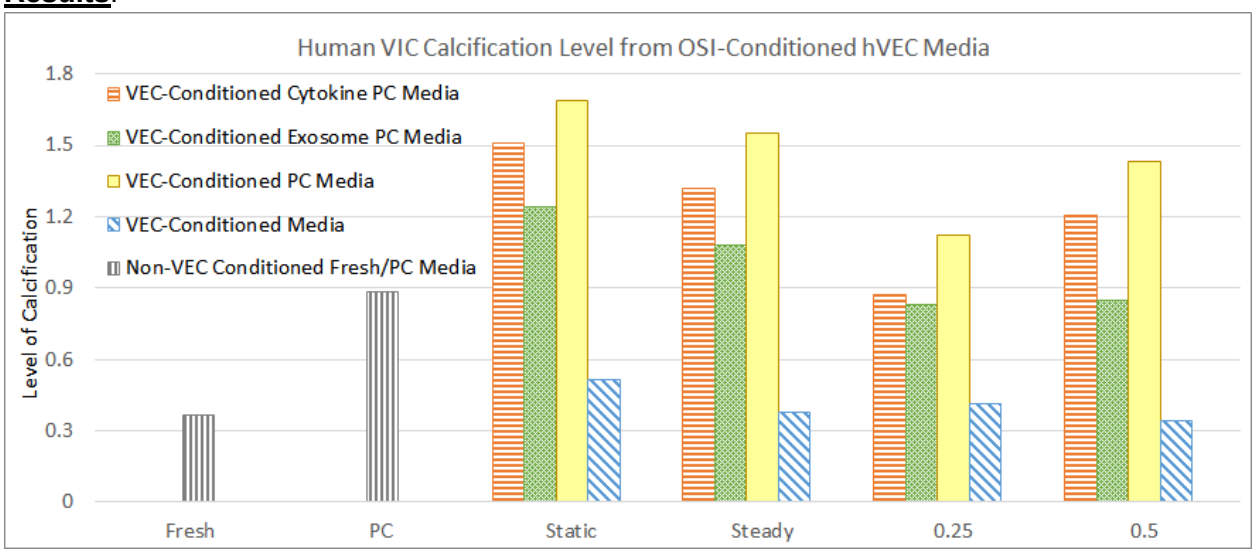
Title:
Valve Endothelial Cell Secretions Augment Calcification by Valve Interstitial Cells

Authors:
Chia-Pei Denise Hsu[1], Joshua Hutcheson[1], Sharan Ramaswamy[1]
[1]Department of Biomedical Engineering, Florida International University, Miami, FL 33174

Introduction:
The biomechanical functions of the aortic heart valve rely on the action of its thin leaflets that open and close over the course of a cardiac cycle. A monolayer of valve endothelial cells (VECs) resides on the outer surface of the aortic valve leaflet. Deeper within the leaflet are sublayers of valve interstitial cells (VICs). Valve remodeling often involves paracrine signaling between VECs and VICs, and diseases such as valve calcification can result from abnormal communication between these cells. VECs are known to respond to hemodynamic stimuli, and studies have shown that VECs exposed to disturbed flow can lead to proinflammatory phenotypic changes and endothelial-mesenchymal-transition [1]. In addition, valve endothelial dysfunction can then lead to the phenotypic switching of quiescent VICs to osteogenic VICs, leading to valve calcification. However, the signaling pathway from alterations in fluid oscillations to valve calcification remains unclear. In the present investigation, we used the oscillatory shear index (OSI) as a parameter to quantify the temporal changes in shear stress direction. OSI ranges from 0 (no oscillations) to 0.5 (equal forward and reverse fluid flow) [2]. We therefore examined VIC calcification in response to equal amounts of pro-calcific media (PC) with ultracentrifuged exosome and cytokine conditioned media from VECs cultured under different OSI flow environments.

Methods:
Human VECs and human VICs were purchased from Lonza Bioscience (Basel, Switzerland) and expanded in T75 flasks. VECs were seeded for 24 hours at $2.0x10^5$ cells per channel in 24-well Bioflux plates (Fluxion Biosciences, Inc. Alameda, CA) consisting of 8 microfluidic channels per plate. The VECs were conditioned for 48 hours in the Bioflux shear stress cell assay system at an average shear stress magnitude of 1 dyne/cm^2 under the following OSI conditions: static (0 OSI/no flow), steady flow (0 OSI/steady flow), 0.25 OSI (moderate oscillation), and 0.50 OSI (full oscillation). The conditioned media from VEC groups were subsequently collected and ultracentrifuged at 50,000 RPM (~100,000g) for 70 minutes. The cytokine supernatants were transferred into separate tubes while the exosome pellets were resuspended in fresh media. Both groups were subsequently used to culture VICs in 24-well plates with equal volume of fresh PC, with the final concentration consisting of 5% FBS, 1% P/S, 1.8 mM $CaCl_2$, 3.8 mM NaH_2PO_4, and 0.4 units/mL of inorganic pyrophosphate [3, 4]. The VIC culture duration lasted for 1 week with one media change made on day 4. VIC calcification was then quantified by alizarin red staining. Note that the VEC-conditioned media group contained both the cytokines and exosomes, without any further processing of this media.

Results:



Discussion:

VIC culture in the VEC-Conditioned PC media which consisted of both VEC-released cytokines and exosomal secretions, exhibited the maximum amount of calcification across all the groups. VIC culture solely with either the VEC-secreted cytokines or exosomes, reduced the calcification levels, with a greater reduction in the latter group, i.e., exosomes. This suggests that cytokines released by the VECs are primarily responsible for inducing calcification by the VICs when cultured in PC media. To develop a 3-dimensional valve calcification engineered tissue model system, these groups will be further investigated after adding VEC-secretions (cytokines and exosomes) to PC media that will subsequently be used for VIC-seeded scaffold culture.

References:

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